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14. ABSTRACT

The National Biocontainment Training Center (NBTC) provides standards-based theoretical and practical training to trainees and professionals preparing for work in biocontainment laboratories where especially dangerous pathogens will be handled. Training is staged and appropriately targeted to requirements for biological safety level 2 (BSL-2), BSL-3 and BSL-4. Structured coursework is designed to prepare trainees to safely manipulate pathogens, including growth, genetic and antigenic characterization, and molecular studies of pathogenesis. Coursework includes both theoretical training and supervised hands-on procedures tailored to meet the specific needs and requirements of the individual trainee.

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W81XWH-09-2-0053 (NBTC1) Annual Report May 2013 – May 2014

INTRODUCTION

The **National Biocontainment Training Center (NBTC)** provides standards-based theoretical and practical training to students and professionals preparing for work in biocontainment laboratories where infectious agents, possibly including especially dangerous pathogens will be handled. Training is staged and appropriately targeted to requirements for biological safety level 2 (BSL2), BSL3 and BSL4. Structured coursework is designed to prepare trainees to safely manipulate pathogens, including growth, genetic and antigenic characterization, and molecular studies of pathogenesis. Coursework includes both theoretical training and supervised hands-on procedures tailored to meet the specific needs and requirements of the individual trainee. Advanced coursework includes hands-on mentored training in the containment laboratories, including consecutive training at BSL3 and BSL4 levels of containment should that be required by the researcher's needs. Specialized training in the handling of laboratory animals is also offered. Sponsored mentored training is available to scientists embarking on a career focused on high hazard pathogens at the BSL4 level through a dedicated fellowship. Fellows in this program work under the close supervision of an established mentor while addressing a research topic of their own choosing. Mentored scientists and fellows progress to full independent access to the BSL4 laboratory only when their mentor and laboratory director are fully confident of the individual's skills and abilities to work independently in this environment.

The NBTC also offers a unique training opportunity for facility operations specialists interested in pursuing a career as a biocontainment operations manager. To the best of our knowledge, this is the only such training program in the world. This program is designed as a series of modules which address specific aspects of the construction, maintenance and safe operation of a biocontainment laboratory. The coursework is tailored to the specific needs of the individual trainee and involves both didactic training and mentored hands-on work using the Galveston National Laboratory (GNL) as its classroom. Over the course of the training period the fellow(s) will be directly involved in the maintenance of the laboratory, decontamination of specific laboratories, monitoring and replacement of filters, fans and control units, understanding the Building Automation System (BAS), and a wealth of other duties routinely seen in the operations of typical biocontainment facilities.

BODY

TATRC's financial support for the NBTC formally began on May 22, 2009 and this submission comprises our annual report for the period **June 2013 to June 2014** for this initiative. The Laboratory Biosafety Training Center at The University of Texas Medical Branch was established and operational prior to receiving TATRC funding; consequently, the course structure and procedures were already in place and allowed the NBTC to implement enhanced training operations almost immediately. From inception to date, the NBTC has provided training to nearly 7,400 participants since program inception through the various courses offered. Below is a summary of the significant progress made over the preceding year (2013-2014) under TATRC support. Funding and accomplishments are organized by each specific aim as they were presented in our original proposal.

Staffing Report.

The NBTC relies on a cadre of highly skilled scientists, engineers and technicians to offer a robust portfolio of training opportunities to trainees and professionals working in the field of biocontainment. Below is a summary of the individuals supported in part by the TATRC award over the past reporting year and their roles in the operation and management of the NBTC.

Dr. Tom Ksiazek. Leadership of the overall NBTC program is provided by Dr. Tom Ksiazek, a veteran of nearly four decades of research and development addressing some of the most dangerous pathogens known to humankind, including the filoviruses, Ebola and Marburg, as well as other causes of viral hemorrhagic fevers such as Crimean-Congo hemorrhagic fever, yellow fever and dengue. Dr. Ksiazek also oversees the BSL4 mentored fellowship program and is responsible for the selection of fellows and for monitoring their progress. As the director of the GNL BSL4 laboratories, he has ultimate responsibility for the individuals working in this space and as such has final approval in determining when an individual has successfully mastered the essential skills needed to safely work independently in the BSL4 environment.

Dr. Anne-Sophie Brocard. Classroom and laboratory training is directed by Dr. Anne-Sophie Brocard, an accomplished virologist and experienced biosafety trainer who has directed the training center courses since their inception. Dr. Brocard provides both theoretical and practical training to trainees and monitors their progress as they develop appropriate skills for work at each level of biocontainment.

Ms. Je T'aime Newton. Dr. Brocard is ably assisted by Ms. Je T'aime Newton, a highly experienced instructor with extensive expertise in biocontainment. Ms. Newton provides specialized training at all levels of containment, but is specifically responsible for preparing trainees for BSL4 investigations, focusing her efforts on the proper care, use and maintenance of the protective "space suits" used in the BSL4 laboratory and other aspects of work in this highly specialized environment.

Ms. Vicki Jones. Ms. Jones is a critical member of the teaching staff who assists as a trainer in both the theoretical and practical training modules.

Ms. Belinda Rivera. Ms. Rivera is a critical member of the teaching staff who assists as a trainer in both the theoretical and practical animal training modules.

Mr. Jason Hardcastle. Mr. Hardcastle is a trainer and assists Ms. Jones and Rivera with the training of students, specializing in *in-vitro* techniques.

Ms. Dee Zimmerman. Ms. Zimmerman is the director of the University's biosafety program and offers guidance in the regulatory requirements for operation of any biocontainment facility.

Mr. Rodrigo Marques dos Santos. Dr. Santos works with ticks and tick borne pathogens, mainly tick-borne encephalitis virus. He has worked with ticks and BSL4 agents within the maximum containment laboratories. He has completed mentored BSL4 training and now has independent access to the BSL4 laboratories.

Ms. Sharon Walters. Ms. Walters serves as the business coordinator for the NBTC and is involved in the outreach program as well as registration process for all external trainees, nationally and internationally.

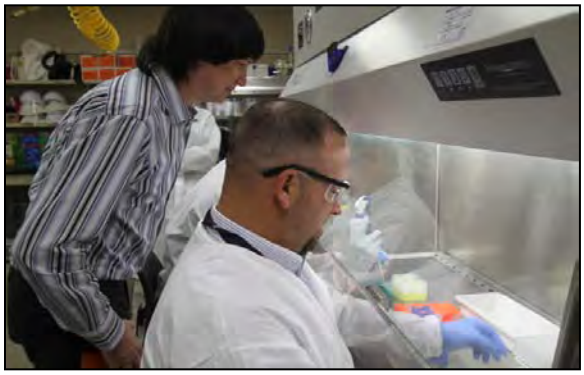
Dr. Janice Endsley. Dr. Endsley is an assistant professor on the UTMB faculty. Dr. Endsley is an expert in tuberculosis and works with XDR-TB. Dr. Endsley will complete her fellowship in June, 2014. She has gained important experience and has mastered skills needed to work in this environment.

Dr. Han Xia. Dr. Xia Joined the NBTC fellowship program in October, 2013 as part of an agreement with the Wuhan Institute of Virology in Wuhan, China – part of the Chinese Academy of Sciences – where she is a research assistant.

Dr. Dennis Bente. Dr. Bente is a BSL4 research scientist with the GNL at UTMB. He is an assistant professor in the Department of Microbiology & Immunology and he serves as a BSL4 scientific mentor for the NBTC. Dr. Bente's research concentrates on understanding how viruses cause disease with special focus on the pathogenesis of Crimean-Congo hemorrhagic fever virus and it's transmission by tick vectors.

Dr. James LeDuc. Dr. LeDuc serves as the principal investigator for the NBTC award and is responsible for programmatic oversight, budgetary issues and reporting requirements. Dr. LeDuc has nearly four decades of experience in the conduct and supervision of research and development activities under biocontainment conditions and he has been intimately involved in the development of national policy in the fields of emerging infectious diseases, bioterrorism preparedness and biocontainment.

Teaching Laboratory Facilities.



The teaching laboratory is a critical asset of the NBTC and it is designed to offer trainees realistic exposure to the conditions and equipment they will typically encounter at each level of biocontainment as they conduct their studies.

At the **BSL2** level, this typically includes a biological safety cabinet where infectious pathogens are handled. *(pictured)*. Biological safety cabinets are used at all levels of biocontainment. The teaching laboratory also includes limited specialized equipment, as well as facilities to manage laboratory waste and storage of pathogens. Training for individuals preparing for work in the **BSL3** laboratory includes a dedicated area where individuals master the donning and doffing of protective gear and its proper disposal.

Those trainees going on to prepare for work in the **BSL4** laboratory have specialized instruction in the care and use of the positive pressure encapsulating ensemble or "space suit" that is worn in the most common type of **BSL4** laboratory in use today *(pictured)*. This includes inspection of the suit for any evidence of leaks prior to use, gaining experience and familiarity in the wearing of the suit, use of compressed air hoses, and training in emergency procedures. In order to provide this training under realistic conditions, the training facility has been outfitted with a breathing air compressor and a mock laboratory where trainees can experience wearing the suit and become familiar with working in this unique environment. Some people discover that they

experience claustrophobic reactions when wearing a suit; this practical, realistic suit training environment allows them to overcome any such reactions under well-controlled conditions, or decide that this work is not a good fit for them.

Laboratory training is conducted in the mock training laboratory with authentic laboratory equipment utilizing non-infectious materials. Entry into the lab is through a double door anteroom with directional airflow and mock pressure monitors. The laboratory has four class II biological safety cabinets, three of which are constructed with see-through panels which allow the trainer to introduce smoke into the BSC to visualize air movement within the BSC. One BSC class II cabinet also has see-through panels and **BSL4** air connections ports. Two of the BSC cabinets are equipped with cameras inside that allow for remote demonstrations. The laboratory itself also has two cameras installed for remote demonstrations. The laboratory has two class III biosafety cabinets, incubators, centrifuges, bench top autoclave, refrigerator, -80° freezer, dunk tank, and general laboratory equipment to allow for mock BSL2 to BSL3 and -4 laboratory work. The laboratory is equipped with a compressor and air lines for practice in the suit check examination and use of BSL4 suits within that facility prior to the trainee entering the active BSL4 laboratories. The laboratory also has multiple airline drops allowing the trainee to work throughout the laboratory with the ability to connect and disconnect airlines as needed.

Overview of NBTC Training.

The NBTC provides a series of training modules involving a mixture of didactic instruction and hands-on training to be carried out within an existing mock BSL3/4 training laboratory that is located within the Environmental Health and Safety Office space in the Materials Management Building on the UTMB campus. The intent of the NBTC is to promote good laboratory techniques and safe procedures to be used at all biosafety levels, and to provide consistency in research practices. The program is designed to ensure that all training attendees have the same general biosafety training at BLS2 and -3, and if necessary BSL4, prior to entering an active biocontainment laboratory, thus ensuring that high safety standards are observed and good practice is consistently pursued.

BSL2 and BSL3 Training.

The BSL2 and -3 training provide a multi-phased approach:

- the assessment phase
- the training phase
    - theory
    - hands-on practicum
- final assessment

Each trainee begins with an **assessment phase** that includes a written test focusing on safety related topics, hands-on skills related to protocols based on their research using appropriate biosafety practices and procedures. During the assessment the trainer does not intervene as he/she notes both safety and scientific techniques employed by the trainee. This allows for the determination of experience and level of training that will be required for each trainee. Once the initial assessment is completed the results are reviewed with the trainee, the specific areas of training focus are identified. A written report is then sent to the trainee and their principal investigator or supervisor.

The **training phase** includes a theoretical class which covers the following topics:

- BSL1 through -4 standard microbiological practices, special practices, safety equipment and laboratory facilities.
- Personal protective equipment, types of respiratory protection, gloves, gowns, use and disposal.
- Proper use of laminar flow biological safety cabinets (BSC), how the cabinet functions, monitoring the BSC functions, setting up the work field, decontamination prior to and after work, and spill response in the BSC.
- Procedures with the potential for creating infectious aerosols, recognizing aerosol producing devices and learning how to mitigate and control aerosol production.
- Emergency procedures, spills in the laboratory, BSC, centrifuge, incident response, cleanup, first aid, reporting procedures, and medical emergencies in the lab.
- Waste management, types of disinfectants, types of waste generated, the differences in disinfection, decontamination and sterilization.
- Introduction to select agent rules and NIH-Office of Biotechnology Activities guidelines.

The **hands-on practicum** during the training phase compliments and reinforces the theoretical class and allows the trainee to experience different scenarios in a non-hazardous environment. It also allows the trainer to observe, advise and correct the trainee's techniques in the laboratory relative to safety as well as scientific issues (e.g. contamination of cultures). The practicum is specific to biosafety and agents to be used (e.g. bacteria, parasites, viruses) by the trainee. This approach also allows the use of specific protocols or facility specific practices that the trainee brings with them. Emergency response and spill mitigation training allows the trainee to visualize and respond to spills and contamination with the use of florescent dye and breakable training lab ware.

The **final assessment** is identical to the initial assessment, with a written exam and hands-on skills assessment. Once the trainee has completed and passed the final assessment, the trainee is provided with a certificate of training for the biosafety level they completed. A report is sent to the principal investigator, the trainee and the laboratory director.

Animal BSL2 (ABSL2), Animal BSL3 (ABSL3), Animal BSL4 (ABSL4) and Non-Human Primate (NHP) Training.

An animal biosafety training program was developed and instituted based on the same concepts as the BSL3 training program. Trainees must have completed BSL3/BSL4 training before commencing ABSL3/ABSL4 training. ABSL2 and NHP training are completed as required by animal specific protocols. Following completion of an animal training program, trainees can return to the program to enhance their animal handling skills for specific techniques and species. All animal use is approved by our institutional animal care and use committee, and our animal holding facilities and procedures have been approved by AAALAC. All aspects of our animal training program have been reviewed and approved by the USAMRMC Animal Care and Use Review Office (ACURO).

The ABSL training phase includes a theoretical class which covers the following topics:

- Personal protective equipment, types of respiratory protection, gloves, gowns, use and disposal.
- Proper use of the BSC, how the cabinet functions, monitoring the BSC functions, setting up the work field, decontamination prior to and after work and spill response in the BSC.
- Procedures with the potential to create infectious aerosols, recognizing an aerosol producing devices and learning procedures to mitigate and control aerosol production.
- Emergency procedures, spills in the laboratory or in the BSC, incident response, first aid, reporting procedures, and medical emergencies in the lab.
- Waste management, types of disinfectants, types of waste generated, the differences in disinfection, decontamination and sterilization.
- The hands-on practicum compliments and reinforces the theoretical class and allows the trainee to experience different scenarios in a safe working environment. It also allows the trainer to observe, advise and correct the trainee's techniques in the laboratory relative to safety as well as animal handling. The practicum is specific to biosafety and animal species to be handled. This approach to training also allows the use of specific protocols or facility specific practices the trainee brings with them.

In the third phase, the final assessment includes a written exam and a hands-on skills assessment. Once the trainee has completed and passed the final assessment the trainee is provided with a certificate of training for the biosafety level they completed.

BSL4 Training.

BSL4 training rests upon a firm adherence to the principles and specific practices of safe BSL-3 research. This practice prevents an over-reliance on the BSL4 suit as a primary means of personal protection, and makes the suit an operationally redundant means of personal protection, significantly enhancing overall containment. Accordingly, individuals who are selected for BSL4 training will have completed training at BSL3 and have been approved for independent access to the BSL3 laboratories. These individuals would then complete the BSL4 modular training.

Specific Aims.

<u>Aim 1: To provide standards-based, high containment laboratory safety knowledge</u>.

Standard training activities for UTMB staff, trainees and investigators from outside the University have been in place throughout the year. The number of individuals trained at each level is summarized in **Table 1** below and the associated figures. As summarized above, the topics typically covered in this introductory training include the principles of basic safety precautions in the laboratory, routine rules and regulations designed to protect the individual and environment from accidental contamination by an infectious microbe, and the care and use of the biological safety cabinet. Also covered are the appropriate procedures of clean-up following a spill, decontamination procedures, principles of the care and use of autoclaves and other essential equipment.

<u>Aim 2: To provide standards-based, high containment laboratory hands-on training.</u>

Training offered under this aim is directed at providing trainees with practical, real-world training in the laboratory setting. Course content is tailored to include those procedures and the use of specific equipment likely to be encountered by the trainee in their routine work. Thus, those destined to work in a virology laboratory may focus on those protocols most appropriate for use in a virology laboratory as opposed to those typically used when working with bacteria. There are, of course, common practices used in any BSL-2 laboratory and training on the safe conduct of these procedures is the foundation of this course. Training typically is undertaken in small groups of only one or two individuals, allowing for intense interaction between the instructor and trainees. By actually doing the procedures essential to their day-to-day laboratory work under the close supervision an instructor (with the use of indicator dyes that allow clear recognition of contamination), the trainee quickly grasps the key teaching points and rapidly masters safe laboratory practices. Training is offered in the state-of-the-art training laboratory described above.

The number of individuals trained under Aim 2 for the NBTC is summarized in **Table 1.**

<u>Aim 3: To provide topic-specific training.</u>

Autoclave Operations. A routine requirement for all persons working in containment laboratories is the need to be able to properly operate autoclaves. A dedicated training session is offered to all individuals as a separate element of their orientation to the GNL, and this training is offered to individuals working in other laboratories using the same or similar equipment.

Aerobiology. The GNL contains aerobiology laboratories at both the BSL3 and BSL4 levels of containment. The BSL3 facilities were fully commissioned and approved for use by the CDC and USDA in early 2010, and the GNL BSL4 facilities were approved for full operations in May 2010 with operations beginning in September 2010. These facilities are highly complex and require specialized training not only in the operation of this sophisticated equipment, but also in the proper care and

handling of the laboratory animals that will be experimentally infected. Aerobiology personnel receive instruction and specialized training about how aerosols are generated, the safety precautions in place in aerobiology laboratories and how to quantitate virus in aerosol samples.

Gamma Irradiator Training. BSL4 laboratories utilize a number of techniques to inactivate biological materials prior to removal from the containment laboratory. One of the most frequently utilized means of removing materials from the BSL4 laboratory is inactivation by gamma irradiation. In an effort to provide training of individuals that use gamma irradiation at UTMB, a module providing background on radiation biology, radiological and biological safety training, select agent and radiological security training, introductory training on dosage determination and method validation, and practical instruction on the use of the devices used for gamma bombardment was assembled and is offered to staff on an as needed basis.

High Through-Put Screening. We continue to work to develop a systematic training program focused on the safety concerns associated with high through-put screening. We are developing this capability as it represents one of the cutting-edge interfaces between technology and biological sciences and as such potentially creates new challenges for biological safety.

Animal handling skill certificate. Following initial training in ABSL2-ABSL4 individuals return to the training program to continue developing animal handling skills necessary to their research project. Each skill certificate is specific to the species, techniques and biosafety level at which the individual was trained.

Class III Biosafety Cabinet Training. We have in this past quarter developed and taught a new training course that focuses on the use of Class III biosafety cabinet. Class III cabinets have very unique features of design and proper use that differ from the other biosafety cabinets routinely used by researchers. These cabinets are mainly found in aerobiology research and BSL4 cabinet line laboratories.

Aim 4: To provide a mentorship program for scientists working in BSL3/ABLS3 or BSL4/ABSL4 facilities.

Mentored training typically extends beyond a given reporting quarter. The BSL4 mentorship program provides a hands-on training experience under the tutelage of a senior staff scientist (mentor) with guidance from other experienced laboratorians in the real-world environment of a functioning BSL4 laboratory. The experience includes an orientation to the laboratory environment by a senior BSL4 scientist and then working experience with tasks germane to the specific requirement of the person being mentored.

As the GNL laboratory was brought online and the Shope BSL4 laboratory continues to operate, our cadre of experienced BSL4 scientists and scientific staff continues to expand (**Figures 1 & 2**). This is actually decreasing the individual burden of training as it is now spread across a larger number of scientists and staff. The numbers of staff that have passed into and out of the mentorship program is presented in **Table 1**.

An extensive listing of the number of individuals trained during the reporting year can be found in **Table 1**.

Figure 1. Cumulative Number of Independent BSL4 Users – January 2005-January 2014, Galveston National Laboratory

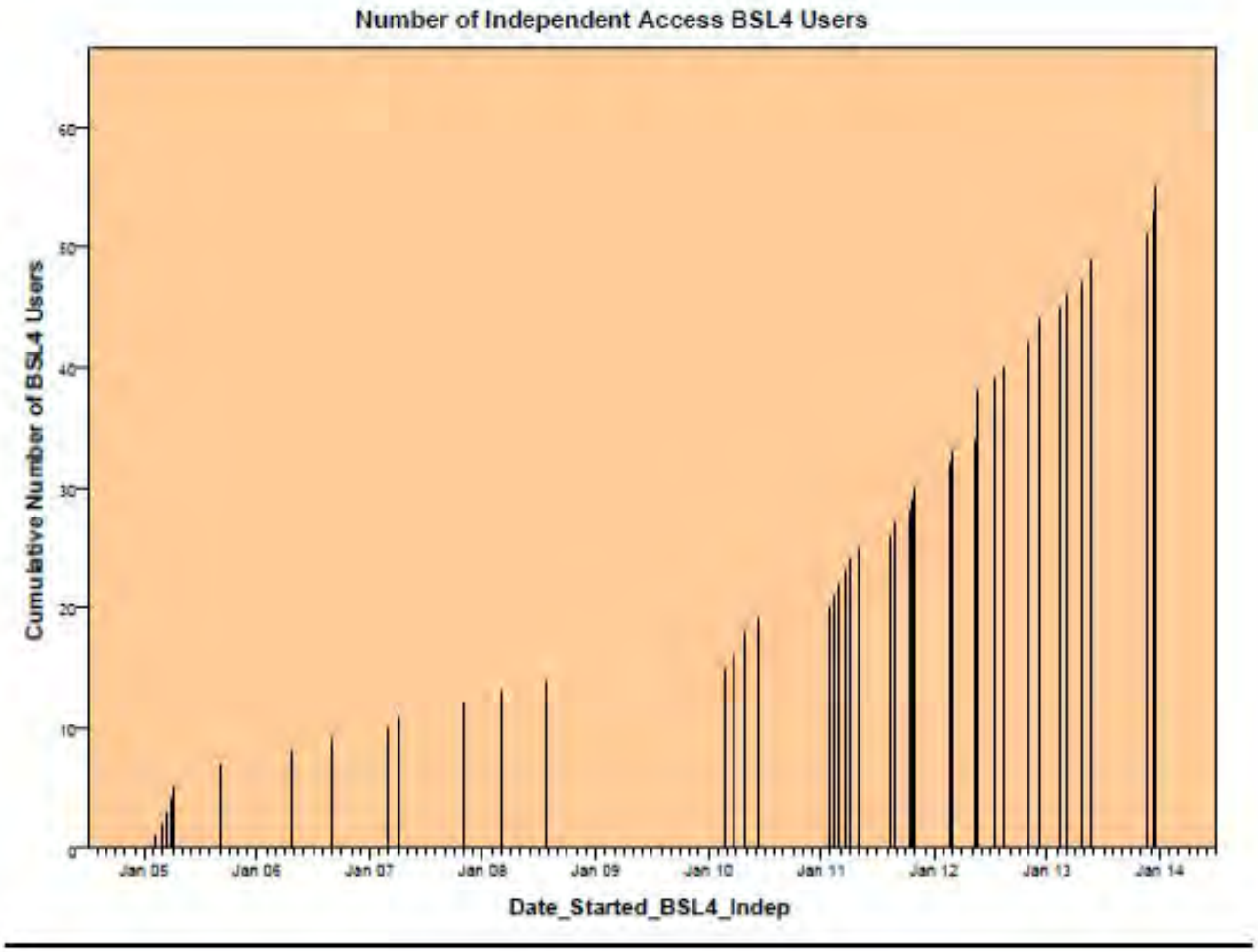
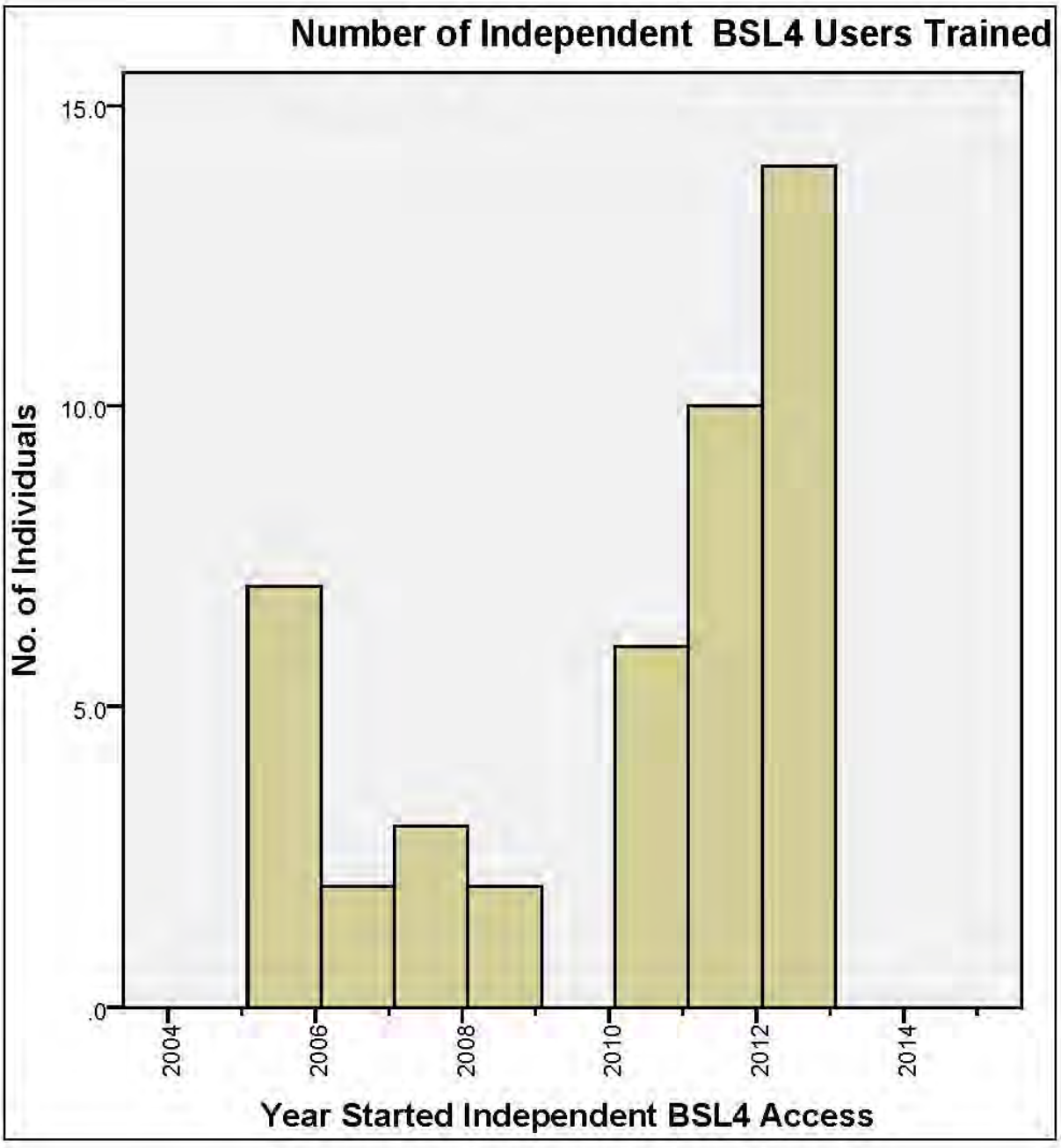


Figure 2. Number of Independent BSL4 Users Trained – 2004-2014, Galveston National Laboratory.



Aim 5: To establish a fellowship program for scientists and facility operations professionals working in BSL3/ABSL3 or BSL4/ABSL4 facilities.

The high and maximum containment fellowship program for scientists is in place. We have two fellows now in training, one fellow who has transitioned from the program to a permanent UTMB faculty position and another who has returned to her native Turkey to lead biocontainment research in that country.

Dr. Endsley' s updates from quarterly reports over the past year detail her progress to May 2014:

Dr. Janice Endsley is a fellow in training in the high and maximum containment fellowship program for scientists for research in the BSL3, ABSL3, and BSL4 laboratories in the GNL. In this role, she provides immunology expertise to investigators working in BSL3 and BSL4 containment, including experimental design, assay performance, and data interpretation. This includes design and interpretation of immunological profiling experiments including performance of Bioplex ELISA, immune recall assays, blood chemistry and blood inflammatory biomarkers, and flow cytometry. As Director of the Flow Cytometry and Cell Sorting Core Facility, she further advises investigators and staff on flow cytometry equipment needs and technical troubleshooting specific to high containment flow cytometers. She is principle investigator of the Tuberculosis Laboratory in the Galveston National Laboratory and has been building a research program in TB Immunology and drug resistance development. In this role, Dr. Endsley is directly responsible for providing hands on, mentored, training for all personnel working with BSL3 level mycobacterium including *Mycobacterium tuberculosis (M.tb)* and performance of mycobacterial infections in animal models in the ABLS3 housing facilities. This includes laboratory technicians, safety officers, and graduate students in need of *M.tb*-specific or general BSL3/ABSL3 training. Through collaboration she is contributing to efforts to develop novel diagnostic platforms to detect drug resistant TB and identification of novel antimicrobial compounds with antibacterial activity against TB (Vijayakumar, et al., Dec. 2013, *Tuberculosis*). She has also developed, optimized, and/or adapted several new techniques for study of bacterial lung infections (*M.tb*, *B. pseudomallei)* in the BSL3/ABSL3 including confocal microscopy-based bacterial viability detection, *in vivo* imaging of bacterial infection and dissemination kinetics in live animals, multi-color flow cytometry, multi-plex ELISA, and *in vitro* infection assays.

Dr. Rodrigo Ivo Marques dos Santos fellowship update, May 2014:

Dr. Santos is a post-doctoral fellow from Brazil working in the laboratory of Dr. Saravanan Thangamani on tick-transmitted pathogens. Under the NBTC fellowship, Dr. Santos completed his BSL3 training and has now gained independent access into the GNL BSL4 laboratories where he is working on the mechanisms of virus transmission by infected ticks. Some of the most dangerous vector-borne diseases are transmitted by ticks, and the Galveston National Laboratory is unique in its ability to maintain and conduct experiments using live vector ticks and the pathogens then transmit under biocontainment conditions, including BSL4. Tick-borne encephalitis (TBE) is an often fatal viral disease transmitted to humans by infected tick bites. According to WHO, tick-borne encephalitis virus (TBEV), a member of the genus *Flavivirus*, is the most important arthropod-borne virus transmitted by ticks in Europe causing severe human infections. TBEV is endemic over a wide area covering Europe and Asia.

TBEV is divided into three subtypes: Central European (TBEV-CE), Siberian and Far-Eastern (TBEV-FE). *Ixodes persulcatus* is the primary vector to TBEV-FE subtype while *Ixodes ricinus* is the primary vector for the Siberian and TBEV-CE. The Central European subtype produces a biphasic disease in 72% of the cases with a first stage in which unspecific symptoms prevail following by the meningoencephalitic stage with mortality of around 5%. The Far-Eastern subtype typically shows more severe monophasic encephalitis with mortality ranging from 30 to 40%, with high levels of sequelae in the survivors. Infectious agents transmitted by ticks are delivered to the vertebrate host together with saliva at the bite site.

Current literature search shows that majority of studies on tick transmitted infections focuses on later state of tick feeding (>24 hours). However, our lab (and a few other labs) has shown that tick borne viruses are transmitted to the host within the first 3 hours of tick attachment. The molecular mechanisms during the earliest stages of tick transmitted TBEV infection are poorly understood. Our lab's research focuses on unraveling the role of tick saliva in creating an immunologically privileged environment and facilitating TBEV transmission and dissemination. Our lab is uniquely positioned to perform these studies, as we have the state-of-the-art BSL-4 laboratory (Galveston National Laboratory), the expertise on tick biology and also the availability of exotic tick colonies from the GNL's insectary services core. We are also the only academic institution that has the capability to conduct research with ticks in BSL-4 laboratory. To this end we have investigated the cutaneous immune response to TBEV infected tick feeding, and have identified tick salivary genes differentially expressed during the feeding process. Our previous work on tick feeding biology showed appreciable neutrophil and macrophage recruitment to the site of tick feeding. Recently, we have shown that macrophage/mononuclear cells at the tick feeding loci are the primary cells infected with TBEV-CE. Though we had observed the recruitment of neutrophils to the feeding site, we could not identify TBEV-CE positive neutrophils. These background data led us to hypothesize that macrophages are the primary target cells for TBEV-CE, and neutrophils facilitate chemotaxis of macrophages to the site of infection/feeding. We are currently investigating the role of macrophages and neutrophils, *in-vitro*, in TBEV infection and

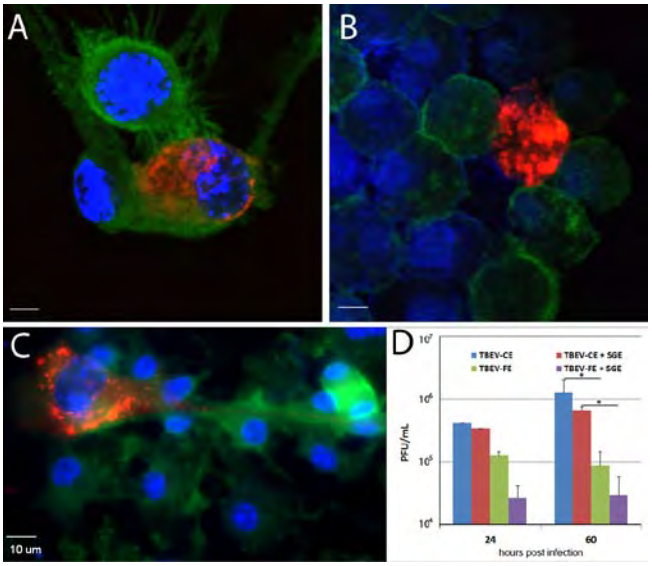


Figure 3: Detection of tick borne encephalitis virus (TBEV) in macrophages. A-C: Immunofluorescence assay for Central European subtype of TBEV (TBEV-CE) and macrophage (F4/80) detection (Fig 1 A); Far-Eastern subtype of TBEV (TBEV-FE) and F4/80 detection (Fig 1B); TBEV-CE and F4/80 (Fig 1C). TBEV-CE and TBEV -FE staining are shown in red (Alexafluor 647), macrophages (F4/80) stains are shown in green (Alexafluor 488), cell nucleus were stained with DAPI (blue). D: PFU assay to determine the susceptibility of macrophages to TBEV-CE and TBEV-FE infection in the presence and absence of tick salivary gland extract (SGE). Statistically significant ($p<0.05$) data are marked by *. Images were taken at the GNL's imaging core facility.

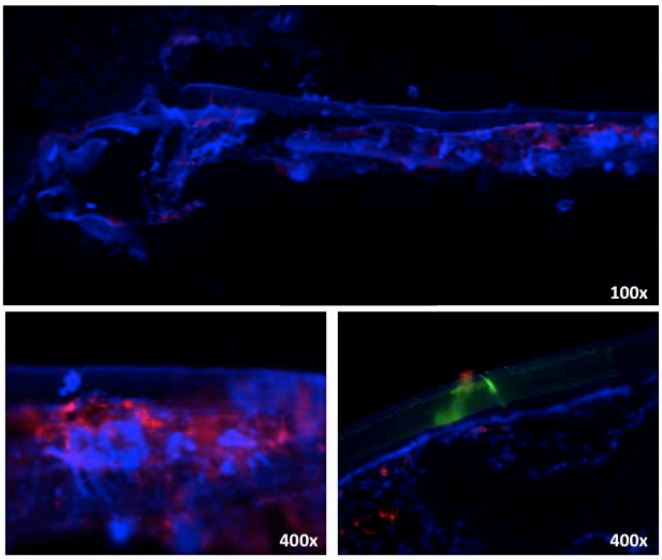


Figure 4: Detection of tick borne encephalitis virus (TBEV) in ticks. Immunofluorescence assay to localize TBEV infection was performed in tick samples using mouse immune ascetic serum generated against Central European subtype of TBEV (TBEV-CE). Cells/tissues with red fluorescence are TBEV-CE positive. These positive tick tissues/cells were used to identify genes that are specifically expressed during the feeding/TBEV infection process. We are currently investigating these genes/proteins for their ability to facilitate TBEV transmission to mammalian hosts.

dissemination and the influence of tick salivary gland extract (SGE). To facilitate this work, we have optimized immunohistochemistry (IHC) and immunofluorescence assay (IFA) protocols for TBEV (Figures 3 and 4) and immune cell detection (Figure 3).

To understand the role of macrophages in TBEV infection and dissemination, macrophages derived from mice bone marrow were infected with TBEV-CE and TBEV-FE with and without tick SGE. At 6, 24 and 60 hours post infection (hpi) supernatant samples were used for virus titration and Bioplex cytokine analysis, while the adherent macrophage cells were used for immune-fluorescence assay (IFA). The IFA showed around 5% of infection in TBEV-CE infected macrophages (shown in red in fig 3A and C) and less than 1% in TBEV-FE infected ones (shown in red in fig 3B). TBEV-CE infected macrophages have macropodia as observed in figures 3A and C, meanwhile TBEV-FE infected macrophages are round-shaped (Fig 3 B). The F4/80 antigen expression (macrophage marker showed in green in fig 3A-C) increases in uninfected cells close to infected ones. It also appears that the uninfected macrophages are grouping around infected cells (fig 3A and C). The cell grouping was not observed when macrophages were treated with SGE. The difference between the titers of macrophages supernatant infected with TBEV-CE and TBEV-FE were statistically significant at 60 hpi (Fig 3D). Significant difference was also found between the supernatant of TBEV-CE and TBEV-FE with previous treatment with SGE at the same timepoint (Fig 3D). The Bioplex analysis indicated that SGE has immunomodulatory effect in IL1a, IL1b, IL6, IL10, MIP1a, MIP1b and G-CSF.

Our data indicates that: (1)TBEV-CE preferentially infects macrophages compared to that of TBEV-FE (Fig 3D); (2) SGE does not affect virus production but can affect the ability of macrophage to recognize infected cells. We are currently investigating the role of neutrophils in the chemotaxis of macrophages to the site of infection, and the role of tick SGE in facilitating this process.

Dr. Han Xia's fellowship program update, May 2014:

Dr. Xia Joined the NBTC fellowship program in October, 2013 as part of an agreement with the Wuhan Institute of Virology in Wuhan, China – part of the Chinese Academy of Sciences – where she is a research assistant. Dr. Xia's research interests include viruses, diagnostic assay methodology for infectious diseases, vaccine research, gene function, genomic and evolutionary analysis and epidemiology. She graduated from the Chinese Academy of Sciences and received her doctoral degree in biochemistry and molecular biology. Since then, she has been working on the development of a mini genome system and epidemiological investigations for Crimean-Congo hemorrhagic fever virus in Western China. She completed an epidemiological survey of CCHFV in Yunnan, China in 2008 (International Journal of Infectious Diseases, 07/2011; 15(7): e459-63). She is interested in a range of emerging arboviruses but mainly in tick-borne bunyaviruses. Her supervisor at the Wuhan Institute remarked: "Every year there are about 20-30 PhD students graduating from the institute. Only very few are retained as junior faculty and she is one of them. Her leaving for the U.S. for two years would be a huge loss to the lab, but we understand that this would be better for the long run and hope for her return upon finishing work in the U.S." While an NBTC fellow, Dr. Xia has been working in the lab of Dr. Dennis Bente. During her time in Dr. Bente's lab, Dr. Xia has focused on Crimean-Congo hemorrhagic fever virus (CCHFV) and related nairoviruses.

Dr. Xia completed the facility training workshop under NBTC trainer and engineer Miguel Grimaldo in December, 2013. She is working/training with UTMB's Dr. Naomi Forrester on how to analyze new viruses by next-generation sequencing. Additionally, she has joined a collaborative project currently

underway working with Dr. Eric Bergeron, Special Pathogens Branch, CDC, on a reverse genetics system for Crimean-Congo hemorrhagic fever virus.

Dr. Xia's Scientific Activities to May, 2014:

1. Next-generation sequencing (NGS) for CCHFV in tick and animal tissues

We are sequencing samples from ticks and vertebrate host animals collected at different infection stages, then analyzing the viral genome in tick and animal host to interrogate the CCHFV transmitted between ticks and host animals at the virus population at the molecular level. Dr. Xia developed methods of CCHFV cDNA library preparation from infected ticks. It is often difficult to get high quality viral cDNA libraries from CCHFV infect tick samples, since the host genome is very complicated and the viral load is usually much lower than found in cell culture. She has prepared one sample of CCHFV (strain IbAr10200) from an infected tick for Illumina sequencing and obtained about 4 GB of high quality paired-end data which contained almost 15 million reads. These were aligned with CCHFV reference genome (IbAr10200) and the preliminary results show that some SNPs exist in the CCHFV L segment and M segment (Figure 5 below).

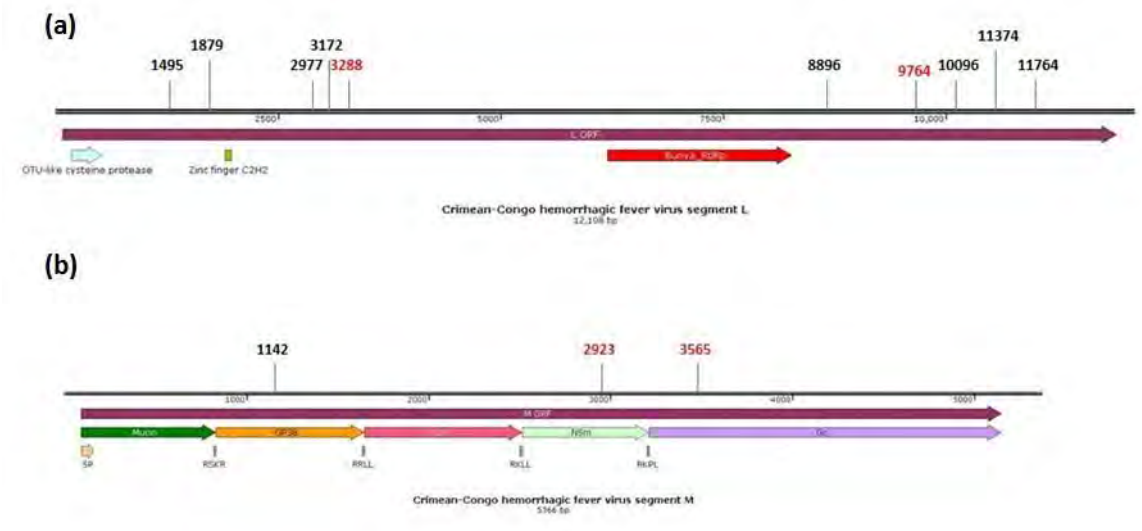


Figure 5. SNPs in CCHFV from an infected tick sample. (a) SNPs positions in L segment. (b) SNPs positions in M segment. The number above the vertical bar indicates the position located in genome; black indicates no change in the coded amino acid; red indicates amino acid changes.

Our plan is to prepare other samples for Illumina sequencing, which includes CCHFV viral tissue culture stock (IbAr10200) and from ticks which have been infected with CCHFV (IbAr10200) for about one year. She will then compare and analyze the data.

2. Nanoluc labeled recombinant CCHFV

Nanoluciferase (Nanoluc) is a newly developed small monomeric luciferase reporter with the brightest bioluminescence reported to date. We hope to create a recombinant CCHF reporter virus (Based on rescue system from our collaborator, Dr. Burgeron, CDC) with Nanoluc which will then be used in our study of viral replication and pathogenicity *in vitro* and *in vivo*. We plan to insert Nanoluc into 3

different positions of M segment (see Figure 6 below), get three transcript plasmids the V0.0 M (SP_Nluc), V0.0 M (Mucin_Nluc) and V0.0 M (GP38_Nluc). To date, Dr. Xia has successfully created the plasmid V0.0 M (SP_Nluc). Our plans is to construct the other two plasmids of M (V0.0 M (Mucin_Nluc) and V0.0 M (GP38_Nluc)) and start the rCCHFV rescue work in BSL-4.

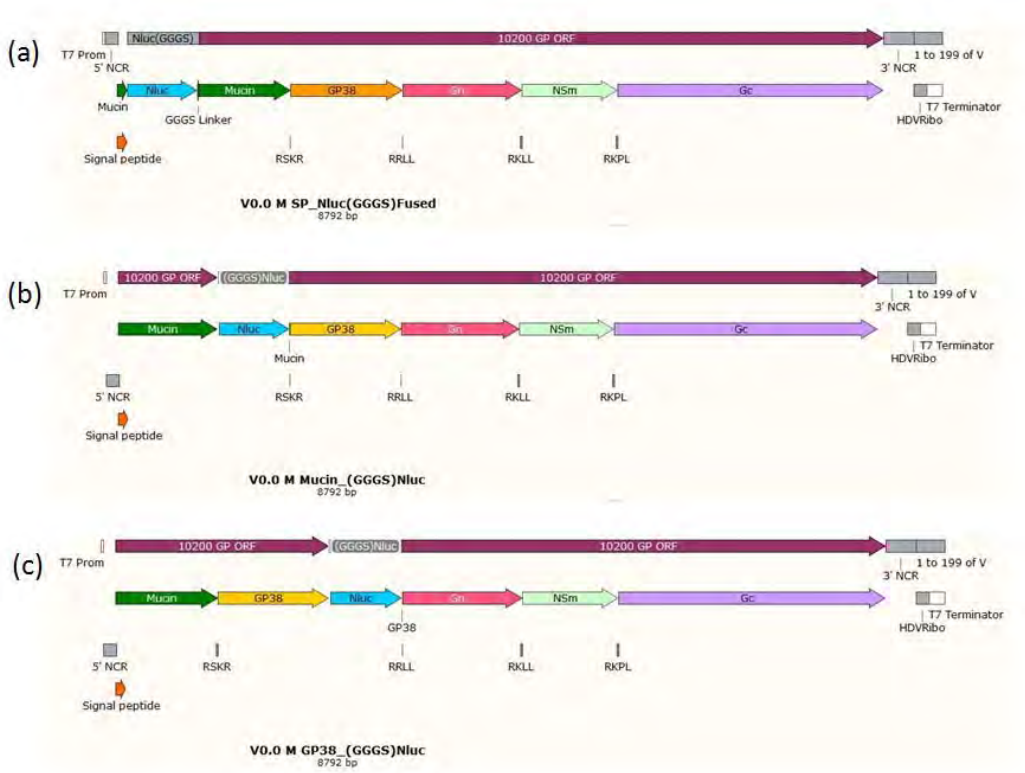


Figure 6. Schematic of the CCHFV M segment fusion with Nanoluc. (a) insert the Nanoluc after the signal peptide, (b) insert the Nanoluc before the RSKR site, (c) insert the Nanoluc before the RRLL site.

3. Mucin and GP38 domain research

Dr. Xia has constructed the eukaryotic expression plasmids which can express mucin with mcherry and GP38 with mcherry and she has constructed the *E.coli* expression system of CCHFV mucin and GP38 domain. Her plan is to use the mcherry marker to detect the position of mucin and GP38 in mammalian cells. She will purify the mucin and GP38 protein to study the role of these two proteins in the progress of viral replication and infection.

A full accounting of her research and fellowship will continue to be included in our companion project report – Award Number W81XWH-11-2-0148, also titled National Biocontainment Training Center.

Dr. Aysen Gargili's fellowship update through May, 2014:

Dr. Gargili completed her fellowship early in this reporting year after spending about 18 months at UTMB. Upon her return to Turkey, Dr. Gargili resumed working at a national biocontainment facility outside Istanbul. While at the GNL, Dr. Gargili accumulated necessary entries and hours in the BSL4 and qualified for independent access. She and her team of researchers conducted studies with the establishment of a CCHF virus infected tick colony in BSL4. Dr. Gargili and her colleagues also designed further infectious studies for the visualization of the virus either in the tick or in infected animals with

real-time imaging techniques. They accumulated critical data on the imaging techniques in live ticks/animals and started to use them in BSL4 for the visualization of the virus in the vector and the host. Her team also collaborated with UTMB researcher Dr. Gustavo Valbuena's team and began to infest humanized mice with infected ticks in BSL4 to see the preliminary results of the infection on human skin. On her return to Turkey, Dr. Gargili has continued her work on CCHF virus and additionally, she has instituted a biosafety training program in Turkey modeled after that in place in the NBTC. To date she has trained in excess of 20 individuals from across Turkey who are working in BSL3 laboratories. This successful example of train-the-trainer amplification of our investments in Dr. Gargili is especially gratifying.

The laboratory containment operations fellowship is unique and is, to the best of our knowledge the only one of its kind. The program sets a new standard for preparation of individuals working in this highly specialized environment. Unlike the fellowship for scientists, where the candidate is likely to already have solid technical skills and will be applying them in the containment environment, most entering facility operators require a more structured course of study. Consequently, we have identified fourteen distinct modules to be covered over the course of the fellowship. These structured modules cover basic microbiology, provide an overview of biosafety and biocontainment principles, construction methodologies specific for each level of containment, risk assessments, select agent regulations, formal Good Laboratory Practices, annual certification of laboratories, testing of HEPA filter housing and filters, air balancing procedures, building automated systems and engineering controls, effluent treatment systems, autoclaves care and use, decontamination procedures, biosafety cabinet certification, and laboratory operations SOP development and record keeping.

During the course of their training, fellows directly participate in each of these activities, as well as are personally involved in the planned shut down and decontamination of containment suites, validate decontamination, and conduct and oversee maintenance activities.

Aim 6: To provide training in Biosurety and Biosecurity for both leaders of biocontainment laboratories and for staff working in such facilities.

Over this past year of support we have actively participated in the national dialogue over the critical elements necessary for an effective program in biosurety and biosecurity of biocontainment laboratories. This remains an evolving field, with guidelines still being developed and a legal foundation yet to be established. As these issues are being debated nationally, we have continued to provide our expert opinion through the American Society of Microbiology and other organizations at the fore of these discussions. We have also participated in ongoing discussions on these topics hosted by the National Science Advisory Board for Biosecurity (NSABB) and the Trans-Federal Task Force on Optimizing Biosafety and Biocontainment Oversight.

Biosurety and biosecurity are currently covered in our theoretical training sessions, and we are in the process of developing a structured course to more specifically address biosurety and biosecurity in depth. We anticipate posting this course on our internal website as an educational tool to augment our more formal training opportunities and to complement lectures already offered. Depending upon the success of this internal posting, we will then consider posting the course on our website where it would be available to the general public.

As part of our participation in the national dialogue on biosecurity – and as a service to the research community – we also established a distinguished speaker series. Our *Topics in Biosecurity Symposia Series* has been offered since 2010. Sessions in the series continue to be of great interest to students, faculty and community members and are consistently attended by large crowds drawing positive attention and feedback.

Summary of Biosurety and Biosecurity training activities this past year:

Topics in Biosecurity Symposia Series.

The GNL's *Topics in Biosecurity* seminar series had an active 2013-2014 season with several distinguished speakers including Tom Slezak (Sept 2013), Dr. Roger Breeze (Oct 2013), Dr. Francisco Pinheiro (Nov 2013), Dr. Peter Jahrling (May 2014) and Dr. Michael Osterholm (June 2014).

Session 13 in the series was held September 17, 2013 and featured **Mr. Tom Slezak**. Mr. Slezak *(pictured right)* is a bioinformatics expert with the Lawrence Livermore National Laboratory. His seminar focused on the history of the federal BioWatch program and its relevancy today. His presentation was entitled **"BioWatch After 10 Years: Why was it built and how is it relevant today?"** Mr. Slezak's insight on the inner workings of the BioWatch program and its current status contributed significantly to ongoing discussions regarding research and biosecurity.

Session 14 in the series was held on October 1, 2013. The session featured **Dr. Roger Breeze** *(pictured right with Dr. Jim LeDuc)*. Dr. Breeze is currently the president of the Centaur Science Group in Washington, DC. Centaur Science is a science consulting company specializing in countermeasures against high consequence infections of humans, animals, and plants, some of which are potential biological weapons and others are naturally occurring disease threats in the U.S. and overseas. He also currently advises the Defense Threat Reduction Agency (DTRA) on ways to reduce the biological weapons threat in the former Soviet Union. From 1987 to 1995, he was Director of the USDA Agricultural Research Service Plum Island Animal Disease Center in New York. The title of Dr. Breeze's presentation was **"Looking Ahead in a World of Biological Threats."** His understanding of the future of biosecurity programs, especially those within the U.S. Department of Defense, contributed to a productive session and fruitful discussion.

Session 15 in the series rounded out the fall season and featured **Dr. Francisco Pinheiro** *(pictured right alongside Dr. LeDuc and graduate students/faculty from Brazil who are in residence at UTMB)*. Dr. Pinheiro was an advisor in viral diseases in the program of communicable diseases with the Pan American Health Organization's division of disease prevention and control. During his tenure he helped establish several disease surveillance and monitoring programs, improved the blood

supply in the face of growing challenges associated with blood-borne pathogens in the region, and coordinated the response to many outbreaks of infectious disease including several major dengue outbreaks. Prior to his post at PAHO, he spent a number of years as the director of the Institute Evandro Chagas virus laboratory in Belem, Brazil. The Institute, once a Rockefeller Network Laboratory, is today a non-profit organization which promotes public health in Brazil and is one of the primary labs for virology in the country. Dr. Pinheiro's *Topics in Biosecurity* session was held on November 12, 2013. His presentation, entitled **"Reflections of a Brazilian Virologist on Arboviruses and Other Viruses in Latin America,"** provided a keen historical perspective on virology as well as insight on evolving laboratory biosafety. The session was very well received and, while on campus, he also had the opportunity to meet with several Brazilian scientists working at UTMB.

Dr. Peter Jahrling speaking in topics in Biosecurity, May 2014.

Dr. Jahrling is the chief scientist at the NIAID Integrated Research Facility and the chief of NIAID's emerging viral pathogens section. He is formerly the senior research scientist for the U.S. Army Medical Research Institute for Infectious Diseases (USAMRIID) Frederick, MD where he served as the principal scientific advisor on issues related to medical defense against infectious disease threats. He has authored or co-authored more than 160 scientific publications, chiefly in the areas of filovirus and poxvirus research. Subject matter expert and consultant to numerous agencies including NIH, the National Academy of Sciences, the World Health Organization, the Department of State, the National Security Council, and the CDC. On May 2, 2014 he offered a discussion of the role of the NIAID Integrated Research Facility during his visit to the GNL and spent valuable time meeting with UTMB students and faculty discussing technical challenges of applying modern imaging equipment to infectious disease research.

Dr. Michael Osterholm speaking in Topics in Biosecurity, June 2014.

Dr. Osterholm is director of the Center for Infectious Disease Research and Policy (CIDRAP), director of the NIH-supported Center of Excellence for Influenza Research and Surveillance within CIDRAP, a professor in the Division of Environmental Health Sciences, School of Public Health, and an adjunct professor in the Medical School, University of Minnesota. He is also a member of the Institute of Medicine (IOM) of the National Academy of Sciences and the Council of Foreign Relations. In June 2005 Dr. Osterholm was appointed by Michael Leavitt, Secretary of the Department of Health and Human Services (HHS), to the newly established National Science Advisory Board on Biosecurity. In July 2008, he was named to the University of Minnesota Academic Health Center's Academy of Excellence in Health Research. In October 2008, he was appointed to the World Economic Forum Working Group on Pandemics. On June 3, 2014 he offered an exceptional talk, "Emerging Infectious Diseases: Looking into the Crystal Ball". His talk was very well attended, and he dedicated considerable time to meet with UTMB faculty and students to discuss the interface between emerging infectious diseases and biosecurity.

NBTC Website.

Over the past reporting year, the new NBTC website has continued to develop - www.utmb.edu/nbtc and it has become a home base for the program. Course training schedules and staff listings are continuously added to the website. We also updated our staff listing with new members of the training team. Per its intent, this site serves as a hub of information regarding the Center, our goals, and the resources that the NBTC makes available to the biosafety and research communities.

Onsite/Offsite training and related updates from the past reporting year.

External Training Highlights (May 2013- May 2014). Of note during this reporting quarter was NBTC training provided to individuals at the following domestic external institutions/corporations:

May 2013 to February 2014:

- ABSL2 training:
  - Baylor College of Medicine and MD Anderson Cancer Center - 2 veterinarians
  - University of Houston - 2 individuals
- ABSL3 training:
  - Baylor College of Medicine and MD Anderson Cancer Center - 2 veterinarians
  - University of Texas El Paso - 17 individuals for theory, 4 for practicum - training done at UT El Paso
  - University of Texas Southwestern - 14 individuals for theory, 3 for practicum - training done at UT Southwestern
- BSL2 training:
  - NuAir – 1 individual
  - University of Houston – 2 individuals
- BSL3 training:
  - Tulane University Primate Center - 1 individual
  - Baylor College of Medicine - 2 individuals
  - Oklahoma State University – 1 individual
  - Fred Hutchinson Cancer Research Center – 1 individual
  - Dept. of Defense Contractor/Booz Allen Hamilton – 1 individual
  - Arizona State University – 1 individual
  - University of Texas Southwestern - 17 individuals for theory, 8 for practicum - training done at UT Southwestern
  - Georgia Southern University - 1 individual for theoretical and practicum

- PAPR training:
    - Tulane University Primate Center - 1 individual
    - Baylor College of Medicine - 2 individuals
    - Oklahoma State University – 1 individual
    - Fred Hutchinson Cancer Research Center – 1 individual
    - Dept. of Defense Contractor/Booz Allen Hamilton – 1 individual
    - Arizona State University – 1 individual
    - Georgia Southern University - 1 individual

External training and instruction was also provided during the 56th Annual meeting of the **American Biological Safety Association (ABSA) Conference** which was held in Kansas City, Kansas (October 17-23, 2013). Several of the NBTC trainers led pre- and post- conference sessions for domestic and international conference attendees. These sessions (and NBTC trainers involved) included:

Ms. Belinda Rivera taught an eight-hour class on **"Advanced Principles and Practices of Working in an ABSL3."** Twenty-two individuals participated in the course. The following is a course description and a list of course objectives.

This course introduced information to individuals that are currently working, plan on working, or audit ABSL-3 facilities. Working in an ABSL-3 facility has unique hazards. Personnel working in these facilities need to be informed of these hazards and trained to work safely and appropriately with the species being handled. Topics will include personal protective equipment (PPE), animal handling procedures, husbandry procedures, caging options, waste management, and emergency response procedures. Institutions with ABSL-3 facilities need to involve safety personnel, animal care staff, and researchers to ensure proper work procedures and safety protocols are in place and followed to maintain a safe and productive work environment.

Course Objectives:

- Identify proper PPE and disinfection practices
- Restate the techniques used to manipulate animals safely
- Explain the processes on waste management
- Summarize the knowledge gained regarding emergency and exposure response

Ms. Dee Zimmerman co-taught an eight-hour class on **"BSL3 Operations and Management."** Thirty-eight individuals participated in the course. The following is a course description and a list of course objectives.

This course reviewed the important aspects of the daily operation of a BSL-3 facility from 2 points of view; management of the facility and daily operations. This assumes that you already have a facility built and have all required authorizations to work in it. The course covered the different aspects you need to consider to operate a BSL-3 facility such as approval of a worker, training of workers and maintenance support, occupational health issues, managing waste, maintenance of the HVAC and physical facility, periodic checks on the facility's systems, and emergencies of different types. It also covered daily operations in a BSL-3 such as understanding when it is safe to enter and when you need to evacuate the facility, what to do when the ventilation fails, practical aspects of entry and exit procedures, practical tips on selection and use of PPE, safety considerations within the experimental SOPs, waste handling, facility's cleaning, and how to have equipment repaired or serviced. This course will be conducted in a way that allows for interaction and exchange of experiences between participants and instructors. This course did not cover regulatory aspects from specific countries.

Course Objectives:

- Describe elements of annual verification, emergency response, etc.
- Recognize institutional responsibilities from management to user.
- Describe methods to develop manuals, SOPs, and training.

Dr. Anne-Sophie Brocard co-taught an eight-hour class on **"Advanced Risk Assessment."** The following is a course description and a list of course objectives. Forty-one individuals participated.

In this new, advanced, and interactive course, attendees followed a research project as it evolves over time from basic to multifaceted in vitro and in vivo scenarios based on actual research protocol submissions. Attendees worked together to conduct risk assessments that build upon each other from the discovery of a novel virus to determining the efficacy of experimental vaccines in humans. Multiple systems used in the research progress from cell culture to small animal models using recombinant viral vectors, to macaques, and finally clinical trials. Risk assessments focused on the likelihood of exposure and the severity of consequences from exposure to the multitude of hazards encountered in the increasingly complex research. Course attendees should have a thorough understanding of rDNA work and the linkage between biosafety, risk assessment and risk mitigation.

Course Objectives:

- Analyze complex scenarios by identifying hazards associated with component parts of the plan
- Prioritize risks based on likelihood and consequences of occurrence
- Assess the overall risk and determine mitigation strategies to minimize the risk
- Evaluate mitigation strategies for effectiveness, adjust strategies as warranted

Mr. Miguel Grimaldo co-taught an eight-hour class on **"Advanced BSL3 Facility Operations."** Thirty-seven individuals participated. Following is a course description and a list of course objectives.

This course is a follow up to the BSL-3 Facility Operations and Management course. This advanced course focused on detailed aspects of biocontainment operations of BSL-3, ABSL-3 and enhanced BSL-3 laboratories. It covered risk assessments for biocontainment equipment; facility operations and maintenance SOPs; maintenance personnel training requirements; solid and liquid waste decontamination equipment; procedures, validation and cycle development; area decontamination methodologies; procedures and validations; filtration systems and their validation and testing processes; ventilation control methodologies and ventilation equipment configurations; facility ventilation system testing during normal and failure conditions; test documentation; and record keeping.

Pictured right: Mr. Grimaldo's well attended class at the ABSA meeting.

Course Objectives:

- Explain the facility verification process in detail, including recommended test methodologies
- Restate the training requirements for facility personnel accessing the biocontainment areas
- Identify methodologies for decontamination of areas, equipment, filters, and waste
- Describe elements of biocontainment equipment risk assessments

Ms. JeT Newton, Vickie Jones, Dee Zimmerman and Dr. Brocard facilitated additional courses and attended committee meetings during the course of the conference.

Ms. Jones facilitated the following pre-conference courses: **"Implementation of Programs and Procedures for an Effective Biosurety Program for BSL-3 Laboratories"**, **"The Safe Transport of Infectious and Biological Substances"**, **"Developing and Maintaining Roles & Responsibilities for Risk-Based Access to, Control of, and Accountability for Biological Agents and Toxins"**, and **"Fundamentals of Microbiology and Infectious Disease"**.

Ms. Newton facilitated the following pre-conference courses: **"Managing Infection Risk"**, **"Developing and Maintaining Roles & Responsibilities for Risk-Based Access to, Control of, and Accountability for Biological Agents and Toxins"**, **"Implementing Personnel Security Programs in Biomedical and Microbiological Laboratories"**, **"Bioterrorism Awareness for the Animal Health Community"**, and **"How to Develop an Export Management and Compliance Program Including the I-129 for Deemed Exports."**

Several NBTC trainers play prominent leadership roles within the ABSA organization:

- Ms. Jones is a member of the Pre-conference Course Committee, Membership Committee and Scientific Program Committee.
- Ms. Newton is member of the Membership Committee and Pre-conference Course Committee.
- Ms. Zimmerman is a member of the Pre-conference Course Committee, ABSA-Owned Course Working Group, Membership Committee, and Accreditation Task Force. She has also completed her first year of a three-year term as a council member for ABSA. ABSA's Council is made up of the current ABSA president, the president-elect, the past president, secretary, financial officer and four council members.
- Dr. Brocard is Co-chair of the Professional Development Team. She is also a member of the Membership Committee, Finance Committee, Member Profile Task Force and Biosafety Graduate Course Task Force.

<u>February 2014 to May 2014:</u>

***University of Texas South Western, Dallas, Texas* – BSL3 Training** was provided for faculty and staff working with *Mycobacterium tuberculosis*. Training was arranged by the grant PI and Biosafety officer/Facility Director. A total of 8 people, including the PI, were trained in practices consistent for safe entry, use, and exit of their containment facility. Individuals were provided with a one-on-one training experience while establishing biological safe techniques. This training took place in Dallas, Texas. Trainers: Vickie Jones and Jason Hardcastle.

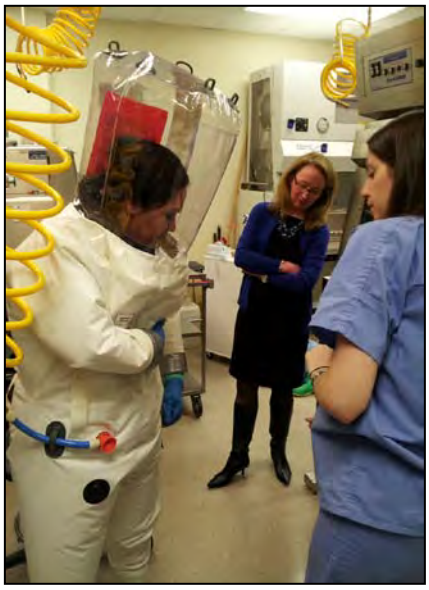
***Food and Drug Administration (FDA-CBER)* – BSL3 training** was provided for faculty and staff transitioning into a newly constructed facility. Training was arranged by the biosafety officer for the entire group of facility users. Theoretical class had 75 participants, and 15 people participated in the full training course. The training took place in the NIH BSL4 training facility in Bethesda, MD. Trainers: Vickie Jones, Belinda Rivera and Jason Hardcastle.

UTMB/FDA-CET – For the past 2 years NBTC trainers have been involved in a joint UTMB/FDA annual course designed to provide FDA inspectors, regulators and personnel involved in oversight of regulated studies with insight on requirements and constraints of conducting regulated research in BSL4 laboratories. The NBTC trainers provided biosafety cabinet technical use awareness for participants in

the course titled "Achieving Data Quality and Integrity in Maximum Containment Laboratories". A total of 16 researchers worked with trainers one-on-one to establish and reinforce safe and aseptic microbiological techniques within a biological safety cabinet in a BSL4 environment. This year the training took place in the NIH BSL4 training facility in Bethesda, MD. Trainers: JeT Newton, Corrie Ntiforo and Vickie Jones.

***Methodist Hospital, Houston, Texas*–BSL2 training.** We provided a highly specialized BSL2 for clinical-pharmacy staff in a ISO Class 7 environment (Compounding Aseptic Containment Isolator). Four individuals directly responsible for the preparation of pharmacy grade intravenous therapeutics were trained in aseptic and fundamentals of biological safety. This training was performed exclusively in a compounding aseptic containment isolator, with special considerations given for the inclusion of vaccines and recombinant material. This training took place in Houston, Texas. Trainers: Vickie Jones and Jason Hardcastle.

Community/External Outreach. During the reporting year distinguished quests both visited the training center and were also provided information on the NBTC through various forums. Highlights of these activities follow:



- In January 2014, staff from the University of Texas System's Office of Federal Relations visited the GNL/NBTC. Included in the visit was Ms. Angela Godby, the UT System's Associate Vice Chancellor for Federal Relations; the newly appointed Dr. Michelle Atchison, Associate Vice Chancellor for Federal Relations; and Jana Lozano, Coordinator for Federal Relations *(pictured right)*. The group was briefed on the NBTC programs and outreach activities. They also had the opportunity to visit the biosafety training mock-laboratory were given a BSL4 demonstration.
- Also in January 2014, NBTC trainer Jason Hardcastle delivered a presentation on the NBTC and his training efforts to the Galveston Rotary Club. This local group of prominent Galveston representatives requested the presentation in an effort to stay abreast of collaborative programs – such as the NBTC – that are underway at the UTMB.

Table 1 below offers a comprehensive summary of all courses offered and number of participants since 2009. Table 2 gives a summary of the international participants receiving training through the NBTC. Figure 7 provides a record of the growth in the number of trainees participating in NBTC courses by year since 2009, and Figure 8 offers a summary of the typical participant by background for the period May, 2005 to December 2013.

Table 1. Summary of training courses offered and number of participants in each course, May 2009 through April 2013.

Training Course/Module	May- Dec 2009	Jan-Dec 2010	Jan-Dec 2011	Jan-Dec 2012	Jan-Dec 2013	Jan 2014	Feb- May 2014	Total
BSL2 theoretical	107	144	251	160	133	3	39	**837**
BSL2 hands-on	64	76	92	117	102	3	29	**483**
ABSL2 theoretical	N/A	1	79	149	284	8	31	**552**
ABSL2 hands-on	N/A	1	40	76	105	11	29	**262**
BSL3 theoretical	45	119	100	135	86	5	89	**579**
BSL3 hands-on	45	101	94	76	57	6	34	**413**
BSL3 mentorship	36	41	24	18	14	3	7	**143**
Class III cabinet theoretical (New course)	N/A	N/A	N/A	N/A	N/A	N/A	2	**2**
Class III cabinet hands-on (New course)	N/A	N/A	N/A	N/A	N/A	N/A	2	**2**
ABSL3 theoretical	29	55	47	73	76	0	8	**288**
ABSL3 hands-on	29	50	46	37	53	1	6	**222**
ABSL3 mentorship	N/A	N/A	38	19	26	2	7	**92**
BSL4 orientation and mentorship	9	28	59	47	41	0	24	**208**
Non-human primate theoretical	16	15	60	41	20	0	9	**161**
Non-human primate, hands-on	N/A	N/A	90	41	28	1	7	**167**
Non-human primate annual refresher	N/A	N/A	37	114	89	0	0	**240**
Animal handling certificates	N/A	N/A	22	75	39	4	21	**161**
Graduate Program	39	36	32	13	18	0	0	**138**
Introduction to Microbiology	18	0	6	4	0	0	0	**28**
Aerobiology	19	5	2	8	0	0	0	**34**
Autoclave	195	27	46	30	25	1	7	**331**
High Throughput Safety training	8	11	3	2	17	3	9	**53**
PAPR (respirator training)	N/A	10	49	52	78	0	9	**198**
ABSA/AfBSA courses	84	175	87	207	181	0	0	**734**
Agent specific training	N/A	N/A	N/A	N/A	186	50	76	**312**
Total trained	**743**	**895**	**1,304**	**1,494**	**1,658**	**101**	**445**	**6,640**

Table 2. International Trainee Summary, 2009 to 2014.

2009				
International Trainee Organization	**Sponsoring Organization**	**Sponsor Funder**	**Training Type**	**Number of individuals trained**
Academia Sinica, Institute of Biomedical Sciences **Taipei, Taiwan ***			BSL3 didactic BSL3 practical	9 9

2010				
International Trainee Organization	**Sponsoring Organization**	**Sponsor Funder**	**Training Type**	**Number of individuals trained**
Institut National d'Hygiène du Maroc **Rabat, Morocco**	World Health Organization	Ministry of Health, WHO, European Union, UNICEF, French Cooperation, International Atomic Energy Agency, Italian Cooperation, WHO COPEP, INSERM – CNRST, CDC	BSL3 didactic BSL3 practical	1 1
GIP WHO Global Influenza Programme **Geneva, Switzerland**	World Health Organization	" "	BSL3 didactic BSL3 practical	2 2
InDRE Instituo de Diagnostico y Referncia Epidemologicos **Mexico**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1
Universidad National Autonoma De Mexico **Mexico City, Mexico**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1
GMI Gorgas Memorial Institute for Health Studies **Panama City, Panama**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1
IOC/FIOCRUZ Laboratório de Virus Respiratórios e do Sarampo **Rio de Janeiro, Brazil**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1
Cantacuzino Institute **Bucharest, Romania**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1
INEI Instituto Nacional de Enfermedades Infecciosas "Carlos G. Malbran" **Buenos Aires, Argentina**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1
Instituto de Medicina Tropical "Pedro Kourí" **Havana, Cuba**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1
Institut Pasteur de Dakar **Dakar, Senegal**	World Health Organization	WHO, Ministry of Foreign and European Affairs, Ministry of Higher Education and Research, Ministry of Health and Sports, The Bill and Melinda Gates Foundation, Wellcome Trust	BSL3 didactic BSL3 practical	1 1
Central Public Health Laboratory Ministry of Health and Population, **Cairo, Egypt**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1
Institut Pasteur in Algeria National Influenza Center Algerian Ministry of Public Health **Algers, Algeria**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1

Chaim Sheba Medical Center Tel Aviv, Israel	World Health Organization	" "	BSL3 didactic BSL3 practical	2 2
UVRI Uganda Virus Research Institute National Influenza Center **Entebbe, Uganda**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1
Ministere de la Sante Publique Laboratoire de Virologie **Yaounde, Cameroun**	World Health Organization	" "	BSL3 didactic BSL3 practical	1 1

2011				
International Trainee Organization	**Sponsoring Organization**	**Sponsor Funder**	**Training Type**	**Number of individuals trained**
Institute Pasteur Casablanca, Morocco *	Moroccan Ministry of Health	World Health Organization	BSL2 Didactic	104
KEMRI Kenya Medical Research Institute **Nairobi, Kenya ***	UTMB institutional funds.	Wellcome Trust	BSL3 didactic BSL3 practical	18 18
University of Ljubljana Ljubljana, Slovenia	State funded institution.	European Union, Structural Funds in Slovenia (European Regional Development Fund, European Social Fund), Innovation & Environment Regions of Europe Sharing Solutions (INTERREG)	BSL3 didactic BSL3 practical	2 2
University of Monterey Monterey, Mexico	University of Monterrey	Privately funded institution.	BSL2 didactic BSL2 practical	1 1
USAMRMC – AFRIMS U.S. Army Medical Research and Material Command - Armed Forces Research Institute of Medical Sciences (a U.S. Department of Defense facility) **Bangkok, Thailand**	U.S. Army	U.S. Department of Defense	BSL3 didactic BSL3 practical ABSL3 didactic ABSL3 practical	3 3 1 1
GMI Gorgas Memorial Institute for Health Studies **Panama City, Panama**	Public institution aligned with the Ministry of Health in Panama.	Research agreements with numerous entities including the U.S. Department of Health & Human Services, Walter Reed Institute of Research and the WHO.	BSL3 didactic BSL3 practical	2

2012				
International Trainee Organization	**Sponsoring Organization**	**Sponsor Funder**	**Training Type**	**Number of individuals trained**
INVEH Institutio Nacional de Enfermedades Virales Humanas "Dr. Julio Maiztegui" **Pergamino, Argentina***	Argentina Ministry of Health	Country of Argentina	BSL3 practical BSL3 didactic Biocontainment engineering	10 27 5
SACIDS Southern African Centre for Infectious Diseases Surveillance (Democratic Republic of Congo, Mozambique, South Africa, Zambia and Tanzania) **Tanzania, Africa***	One Health Commission	Rockefeller Foundation Google Wellcome Trust Nuclear Threat Initiative's (NTI) Global Health and Security Initiative (GHSI)	BSL2 theoretical	12

USAMRMC – AFRIMS U.S. Army Medical Research and Material Command - Armed Forces Research Institute of Medical Sciences (a U.S. Department of Defense facility) **Bangkok, Thailand***	U.S. Army	U.S. Department of Defense	BSL3 practical BSL3 didactic ABSL3 didactic ABSL3 practical NHP training	20 49 26 4 7
University of Monterey **Monterey, Mexico**	University of Monterrey	Privately funded institution.	BSL3 practical BSL3 didactic	1 1
Koc University **Istanbul, Turkey**	Koc University	Privately funded institution.	BSL3 practical BSL3 didactic ABSL3 practical ABSL3 didactic	1 1 1 1
CENETROP Centro Nacional de enfermedades tropicales (linked with other health institutions in Bolivia, Latin America and Belgium) **Santa Cruz, Bolivia***	Pan American Health Organization (PAHO)	Country of Bolivia	BSL3 practical BSL3 didactic	6 6

2013				
International Trainee Organization	**Sponsoring Organization**	**Sponsor Funder**	**Training Type**	**Number of individuals trained**
VIDRL Victorian Infectious Diseases Reference Laboratory **North Melbourne, Victoria, Australia**	State of Victoria	State owned and operated.	BSL4 Training BSL4 Training Biocontainment engineering	2 2 2
SACIDS Southern African Centre for Infectious Diseases Surveillance Sokoine University of Agriculture (Democratic Republic of Congo, Mozambique, South Africa, Zambia, Zimbabwe and Tanzania) **Tanzania, Africa***	One Health Commission	Rockefeller Foundation Google Wellcome Trust Nuclear Threat Initiative's (NTI) Global Health and Security Initiative (GHSI)	Introduction to biosafety course.	17
Technical University of Denmark National Veterinary Institute **Kalvehave, Denmark**	Technical University of Denmark	Technical University of Denmark	Biocontainment engineering	1
The Pirbright Institute **Surrey, United Kingdom**	Pirbright Institute	Biotechnology and Biological Sciences Research Council (BBSRC)	Biocontainment engineering	1

2014				
International Trainee Organization	**Sponsoring Organization**	**Sponsor Funder**	**Training Type**	**Number of individuals trained**
Institute of Medical Biology **Kunming, China**			BSL3 practical BSL3 didactic ABSL2 practical ABSL2 didactic Biocontainment engineering	2 2 2 2 2

Figure 7. Total number of courses administered annually over the history of the biosafety training program – May 2005-December 2013 (Total = 6926). The creation of the NBTC in 2009 has contributed significantly to the exponential growth of the biosafety training program.

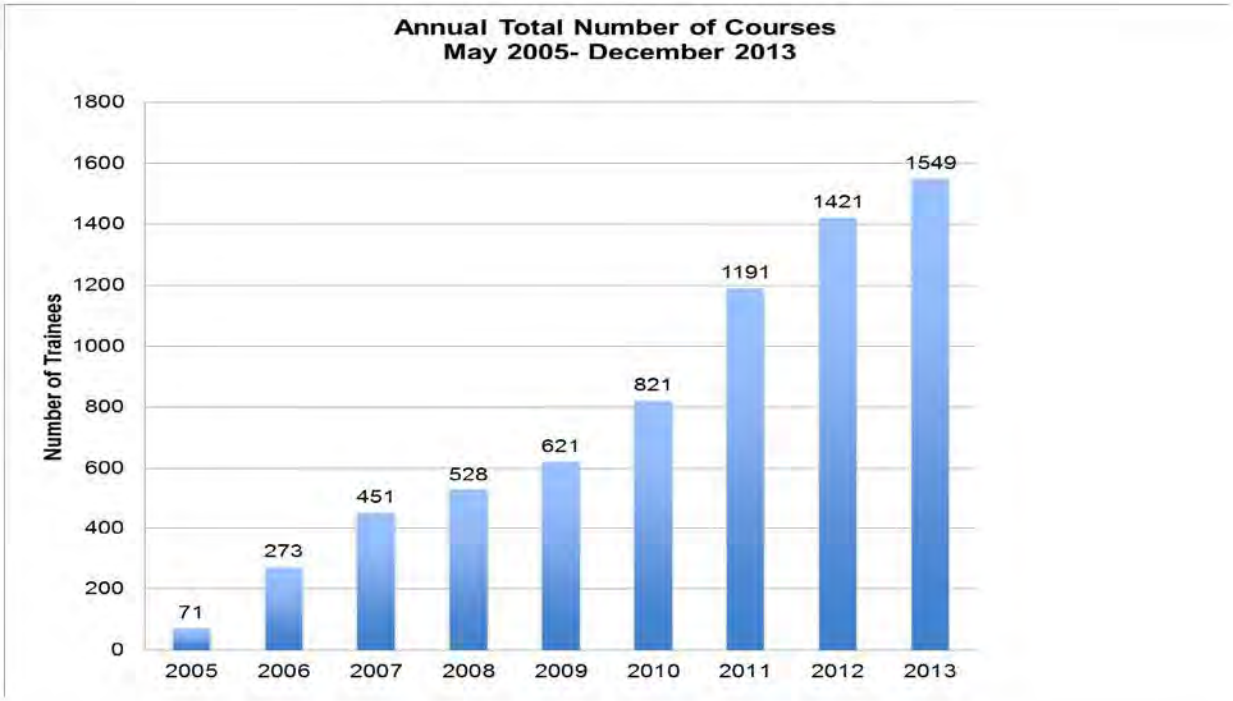
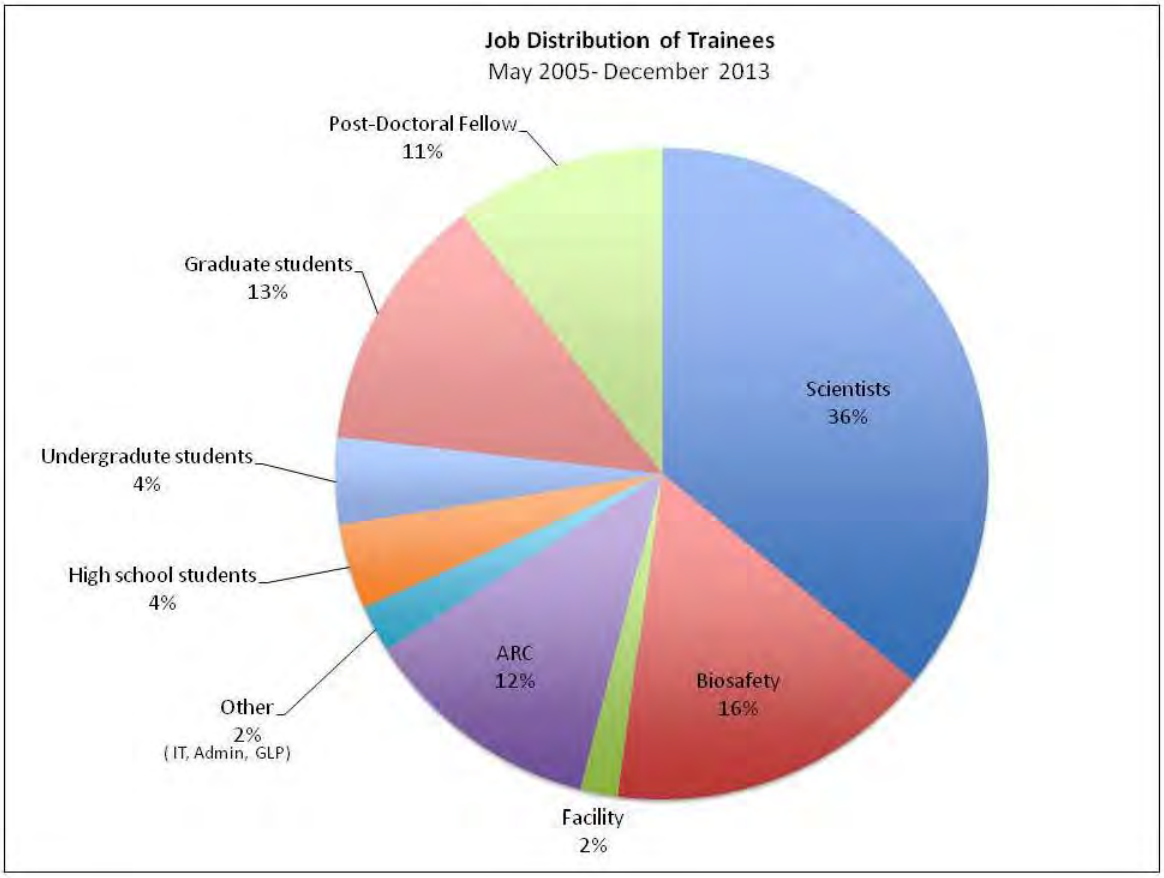


Figure 8. Job distribution of trainees, May 2005 to December, 2013.



KEY RESEARCH ACCOMPLISHMENTS 2013-2014:

- Our comprehensive, standards-based training program was continued and expanded to prepare individuals for work at all levels of biocontainment.
- Advanced, mentored training was provided to select fellows with exceptional skill who desire to expand their research activities to include studies at the BSL4 level.
- Our unique fellowship was continued to provide training to the next generation of containment laboratory facility operations professionals to prepare them to oversee the safe operations of these complex facilities.
- As part of our participation in the national dialogue on biosecurity – and as a service to the research community – we continued to offer our unique seminar series, *Topics in Biosecurity. S*essions offered continue to be attended by large crowds and draw positive attention and feedback. Planning is underway to continue the series with 2014-2015 sessions.

REPORTABLE OUTCOMES 2013-2014:

- Since the inception of UTMB's Laboratory Biosafety Training Program, prior to the TATRC funding award in 2009, nearly 7,400 individuals – both internal to UTMB and external, both nationally and internationally – have been instructed in the safe research and operation in biocontainment laboratories. The vast majority of these trainees have taken part in the program since 2009 when TATRC funding allowed for significant growth of the program.
- Requests for external biosafety training continue to be received in increasing numbers providing clear evidence of the strong demand for high quality training in biosafety and biosecurity.

CONCLUSIONS:

The National Biocontainment Training Center offers a robust and intensive training program devoted to all aspects of biological safety, biocontainment, and biosecurity. This program offers unique, hands-on training to trainees, staff and external partners at all levels of biocontainment, including focused, mentored training in the BSL4 laboratory.

Nearly 7,400 persons have benefited from one or more of these training courses, many of whom are now pursuing graduate education and using these specialized skills in the GNL containment facilities and in other biocontainment facilities around the world. Our intensive, mentored fellowship programs offer opportunities for in-depth training in research under all levels of biocontainment to include BSL4 conditions. We also offer unique training opportunities in containment laboratory operations and maintenance. These fellowships are proving quite successful and are helping address the critical national shortage of well-trained containment laboratory scientists and facility operations specialists.

REFERENCES:

None.